CONTRACT NO.: DAMD17-87-C-7173

TITLE: BIOLOGICAL SIGNIFICANCE OF THE IMMUNE RESPONSE TO HTLV-III/LAV

PRINCIPAL INVESTIGATOR: DANI P. BOLOGNESI

PI ADDRESS: Duke University Medical Center
P.O. Box 2926
Durham, North Carolina 27710

REPORT DATE: March 1, 1989

TYPE OF REPORT: Midterm

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21701-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0168

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Duke University Medical Center 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) P.O. Box 2926 Durham, NC 27710	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-87-C-7173
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63105A; PROJECT NO. 3M2-63105DH29; TASK NO. AD; WORK UNIT ACCESSION NO. 068

11. TITLE (Include Security Classification)

(U) Biological Significance of the Immune Response to HTLV-III/LAV

12. PERSONAL AUTHOR(S)
Dani P. Bolognesi

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Midterm	FROM 8/15/87 TO 2/15/89	1989 March 1	20

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	
06	13	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
RA 1; Cell mediated immunity; Virology; HIV; Vaccine development; Subunit vaccine; Immunology

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

It has been shown that the major neutralizing determinant of HIV lies in the V3 segment of gp120, coded by a dominant, hypervariable region. A "cocktail" of V3 peptides will elicit, reproducibly, high anti-peptide and anti-HIV neutralizing activity for each isolate included in the cocktail.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b. TELEPHONE (Include Area Code) 301-663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

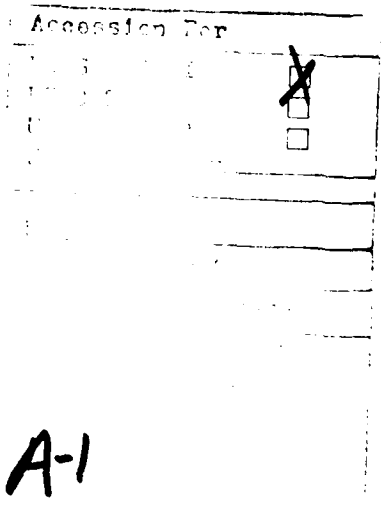
____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

PI Signature — March 1990 Date



A-1

Mid-Term Progress Report

Contract Number DAMD17-87-C-7173

As indicated in the quarterly reports, the research program supported by this grant has been focusing on three aspects of anti-HIV immune responses:

1) Neutralizing antibodies.

2) Antibodies that enhance HIV infectivity.

3) Anti-HIV immune responses in children of infected mothers.

I. Anti-HIV Neutralizing Antibodies.

The work with neutralizing antibodies relates to the role of the third hypervariable region, which is represented by disulfide linked loop within gp120 (V3 loop; amino acids 303-335). Our previous studies demonstrated that this region is a dominant neutralizing epitope of HIV and that response to it was generally variable and specific to the respective isolate. We undertook a study to determine the extent of this variability among a large number of HIV isolates in order to gain insight into which, if any, of the V3 sequences might be more prevalent in the infected population at large.

Resultant studies of over 200 HIV+ human sera revealed a differential seroreactivity to the various V3 sequences with 10% or less reactive to 3B, about 25% to RF, 50-60% to the WMJ2 and SC and 70-80% to the MN peptide (Table I). The more common recognition of the MN sequence has also been noted by others and suggests that the bulk of the virus genotypes in the field show some homology to sequences that fall in the MN V3 domain. In a second approach to this question in collaboration with Dr. Weinhold, in this laboratory and Dr. Putney at Repligen, the V3 sequence from 249 field and prototypic HIV-1 isolates were directly compared following PCR amplification, cloning and sequencing. The results of the study were in agreement with the serotyping approach in that alignments of the V3 sequences with the MN map yielded the lowest overall number of mismatches. The homology was most apparent in the central portion of the V3 region with 44% of the isolates yielding an IGPGRAF sequence at the crown of loop (Fig. 1) in common with the MN isolate. These results suggest that the MN sequence might represent an important vaccine

component and induce a response to a significant fraction of virus genotypes. Consistent with that notion in collaborative studies with T. Palker and B. Haynes, we have found that experimental sera raised to MN V3 peptides induce neutralizing antibody to a number of field isolates (Table 2). Nevertheless, even the MN sequence leaves a large number of isolates with minimal apparent homology and approaches must be devised to overcome that heterogeneity if this region of the virus is to be seriously considered as a component of an HIV vaccine. Studies are currently in progress to determine if there is any cross reactivity between these various loop classes, particularly within the loop crown. The goal of these studies is to arrive at a universal epitope or epitope cocktail that would represent the majority of viruses in the population.

II. Antibody Dependent Enhancement (ADE)

Recent reports of antibody-dependent enhancement (ADE) of HIV-1 infection in vitro have elicited concern regarding the development of HIV-1 vaccines. Whether this phenomenon occurs in vivo is unknown. To explore the possibility that anti-HIV antibodies might enhance the infection of normal human mononuclear phagocytes, we studied the effect of human Ab on HIV-1 infection of human peritoneal macrophages (Mac) and peripheral blood monocytes (Mono). Antibodies tested included fresh sera from 16 HIV-1 Ab-positive patients as well as 3 non-neutralizing human monoclonal Ab's. Blood monocytes were isolated on sequential Ficoll-Hypaque/Percoll gradients; peritoneal macrophages were obtained from women undergoing laparoscopy and isolated by Ficoll-Hypaque sedimentation. The Mono/Mac were further purified by adherence to microtiter plates followed by vigorous washing to remove nonadherent cells. Resultant monolayers (>95% Mono/Mac) were cultured in DMEM with 10% unheated normal human serum. Test antibodies and 1 of 4 HIV-1 isolates were added within 1-5 days of plating. HIV-1 isolates included the lymphotropic strain III_B, the viral-tropic strain Ba-L, and two local field isolates. Antibodies were tested in duplicate at serial dilutions of 10^{-1} thru 10^{-8}. To determine the role of complement, reported to be necessary for the enhanced infection of one lymphocytoid cell line, fresh and heat-inactivated (56C, 30 min) aliquots of sera were tested in parallel. Two to five days post-infection, cells were washed in order to remove sera and fresh culture medium was added. Cultures were then monitored at 1-4 intervals for HIV-1 infection using 3 end points: HIV p24 antigen production, HIV reverse transcriptase

activity and HIV-induced cytopathicity. When tested at low dilutions (10^{-1} to 10^{-2}), all antibody-positive sera neutralized HIV-1 infection. Though this activity was lost at higher serum dilutions, no antibody-dependent enhancement of HIV-1 infection was apparent with any patient sera on either peritoneal macrophages or blood monocytes. The fsinding were consistent with all 4 HIV-1 isolates examined. Heat-inactivation of sera had no significant effect on neutralization or enhancement (Fig. 2). Furthermore, consistent with the established CD4-dependent mechanism of HIV-1 entry, infection of both monocytes and macrophages was blocked by agents that interfere with the binding of HIV to CD4 (soluble CD4 and OKT4a/10Thy5D7) (Fig. 3). These results suggest that antibody-dependent enhancement does not occur in primary human mononuclear phagocytes in vitro. Whether other cell types are targets for ADE mediated HIV infection is currently under study.

III. Anti-HIV Immune Responses in Children.

In infants, the serologic diagnosis of HIV-1 infection is confused by the presence of HIV-1 antibodies transplacentally acquired from the mother. Those infants who are offspring of HIV-1 seropositive mothers, but who are not infected with HIV-1 all lose antibody by 18 months of age. However, only 58% will have lost maternal antibody by one year of age and 78% will have lost maternal antibody by 15 months of age. The HIV-1 infected infants may develop Western blot reactivities distinct from those of their mother as early as three months of age, but some will not produce HIV-1 antibodies for prolonged periods. We have studied a cohort of 36 HIV-1 infected children, all of whom fulfilled the CDC criteria Western blot analysis, of these 25 were clearly positive while 9 were indeterminate and two were negative (Table 3).

Our observations further confirm that children with HIV-1 infection have impaired humoral responses to the virus. It is unclear whether a lack of production of specific antibody, rapid utilization of antibodies, or both, are responsible for the absence of specific antibody bands on Western blot. Twenty-five (73.5%) of the children had evidence of B-cell dysregulation, with elevated or decreased concentration of total IgG, suggesting antibody synthesis problems. All of the children with serum p24 antigen concentration more than 100pg/ml and indeterminate Western blots had no anti-p24 antigen corresponding to declining or absent antibodies to the core proteins p24 and p17, as has been previously noted in children.

Antibody to the transmembrane glycoprotein gp41 was the most consistent Western blot finding in our population of HIV-1 infected children.

The anti-gp41 band also appears to be relatively infrequent in Western blots of sera from non-HIV-1 infected individuals. As a result, the single most reliable band for Western blot interpretation in children is likely to be gp41. However, two HIV-1 infected children lacked anti-gp41 bands.

In summary, children over the age of 18 months who are clinically suspected of having HIV-1 infection and who have a positive ELISA but negative or indeterminate Western blot require additional testing. A serum p24 antigen determination and/or HIV culture should be performed. Alternative diagnostic tests also being evaluated include: detection of the HIV-1 genome in infected cells using the polymerase chain reaction, and in vitro HIV-1 specific antibody production using cultured patient lymphocytes.

We have also examined the same cohort for the presence of anti-HIV antibodies with biological activity. We summarize here the results with antibodies that mediate antibody dependent cell cytotoxicity (ADCC).

ADCC activity was investigated by a series of six hour ^{51}Cr release assays using normal donor lymphocyte effectors and gp120 adsorbed CEM.NKR (natural killing resistant) targets. Twenty-four of 36 children had antibody capable of directing anti-HIV-1 ADCC activities (Fig. 4). Levels of ADCC antibody were generally lower than that previously reported in the adult HIV-1 infected population. When compared to a cohort of 27 HIV-1 infected hemophiliac children, children with vertically acquired HIV-1 infection had significantly lower levels of anti-HIV-1 ADCC activity (Fig. 5). These findings confirm that the humoral defects noted in pediatric HIV infection extend to functional antibody responses including ADCC activity.

Studies are currently in progress to determine the level and specificity of neutralizing antibodies in this cohort and to relate them to those in the mothers. These will be evaluated on viruses isolated from the mother and child respectively.

TABLE 1

		Percent Sera Positive in ELISA
RP 142 (MN)	Y N K R K R I H I G P G R A F Y T T K N I I G (C)	84% (57/68)
RP143 (SC)	N - T T R S - - - - - - - - - - A - G D - - - -	71% (48/68)
RP141 (WMJ-2)	N - V - R S L S - - - - - - - - R - R E - - - -	76% (52/68)
RP139 (RF)	N - T - - S - T K - - - - V I - A - G Q - - - -	37% (25/68)
RP135 (IIIB)	F N T R - S I R - Q R - - - - - - V - I G K - - -	9% (6/68)

TABLE 2

Neutralization Titer on Prototype and Recent Field Isolates

Antiserum	IIIB	MN	RF	18389	18889	18989	19089	19189	20489	21789
T_1Sp10 III_B	1300	<10	<10	40	<10	<10	<10	<10	<10	800
T_1Sp MN	<10	1200	20	200	160	30	270	30	1100	60
T_1Sp10 RF	<10	<10	800	<10	<10	<10	60	<10	<10	<10

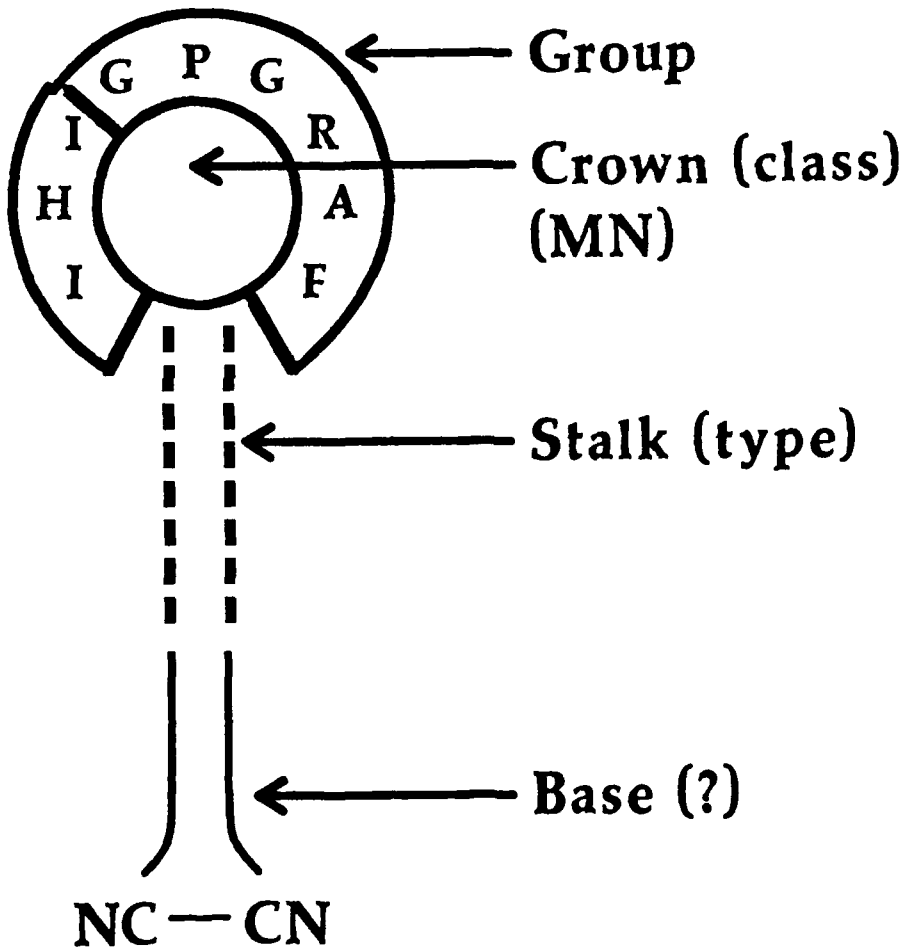
Table 3. Comparison of HIV-1 Infected Children with Varying Western Blot Results

	Western Blot Result			
	Positive	Negative	Indeterminate	Total
Number of Children	25	2	9	36
Mean Age (years)	4.0	0.9	2.0	3.2
Number with Western blot band present				
gp120	17/23	0/1	3/9	20/33
gp41	25/25	0/2	9/9	34/36
p31	16/23	0/1	0/9	16/33
p24	25/25	0/2	0/9	25/36
Number Elisa Antibody Positive	24/24	1/1	9/9	34/34
Number HIV Culture Positive	21/25	1/2	9/9	31/36
p24 Antigen (pg/ml)				
-Mean	24.3	70.0	219.0	78.6
-Range	(0-101.0)	(0-140.0)	(0-428.0)	(0-428.0)
-Median	0.0	70.0	192.6	23.6
Number p 24 Ag (+)	7/23	1/2	8/9	20/34
<400 CD4 cells/mm^3	8/25	1/2	2/6	11/33
CD4/CD8 <1.0	17/25	2/2	7/8	26/35
Elevated Serum IgG	19/24	1/2	4/7*	24/33

*1 patient had hypogammglobulinemia

FIGURE 1

HIV Neutralization Loop



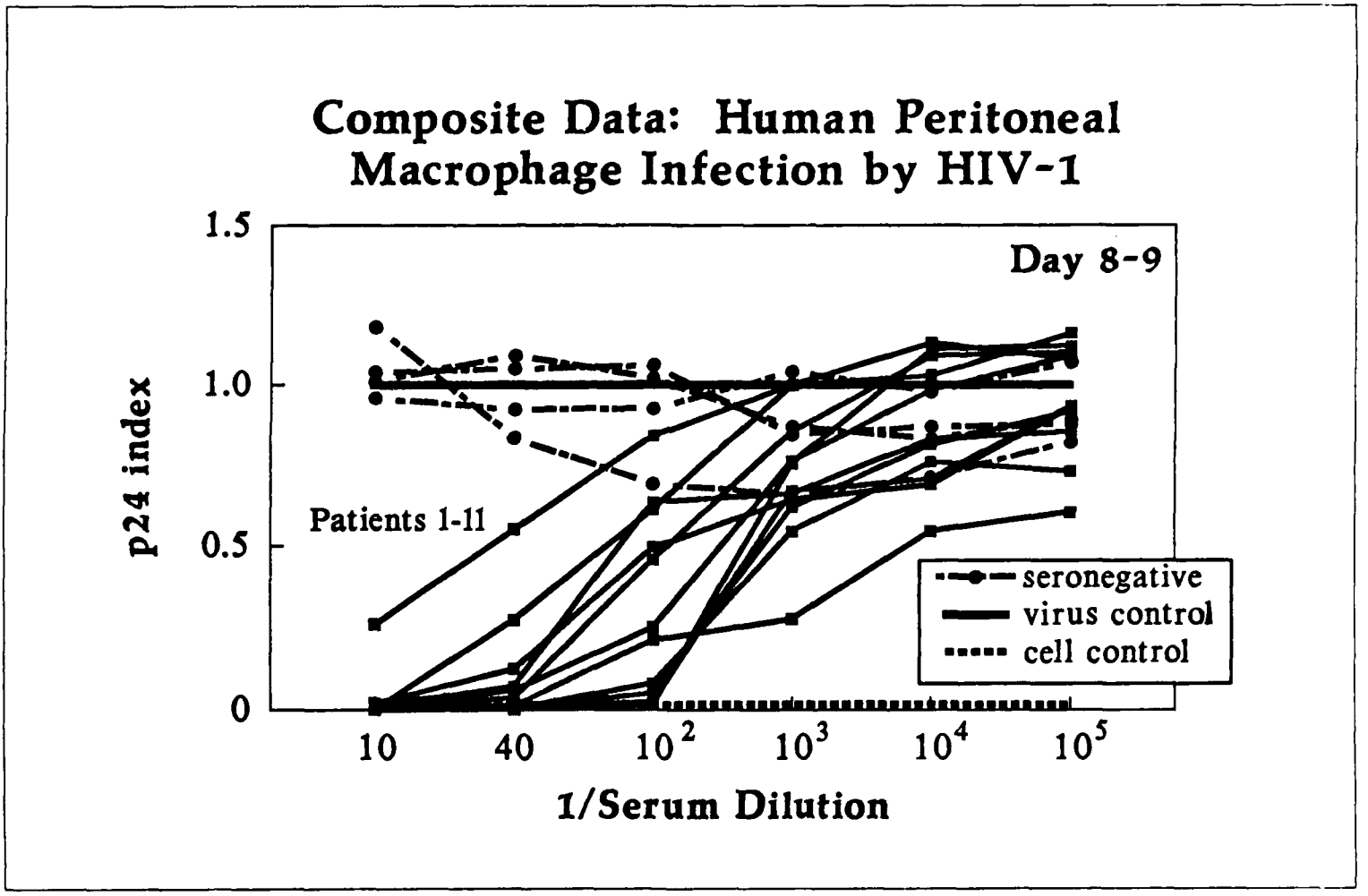


Figure 2. Levels of p24 in the culture supernatant reflect the level of virus liberated. At 8 days post infection, the solid bar represents the p24 levels in infected cultures with no treatment. Addition of antibody resulted in virus neutralization at high concentrations (no p24 release). With progressively lower antibody concentrations, p24 levels rose but did not significantly deviate from untreated cultures at peak levels. Neither the addition of complement nor heating the serum to destroy complement had any effect on the values.

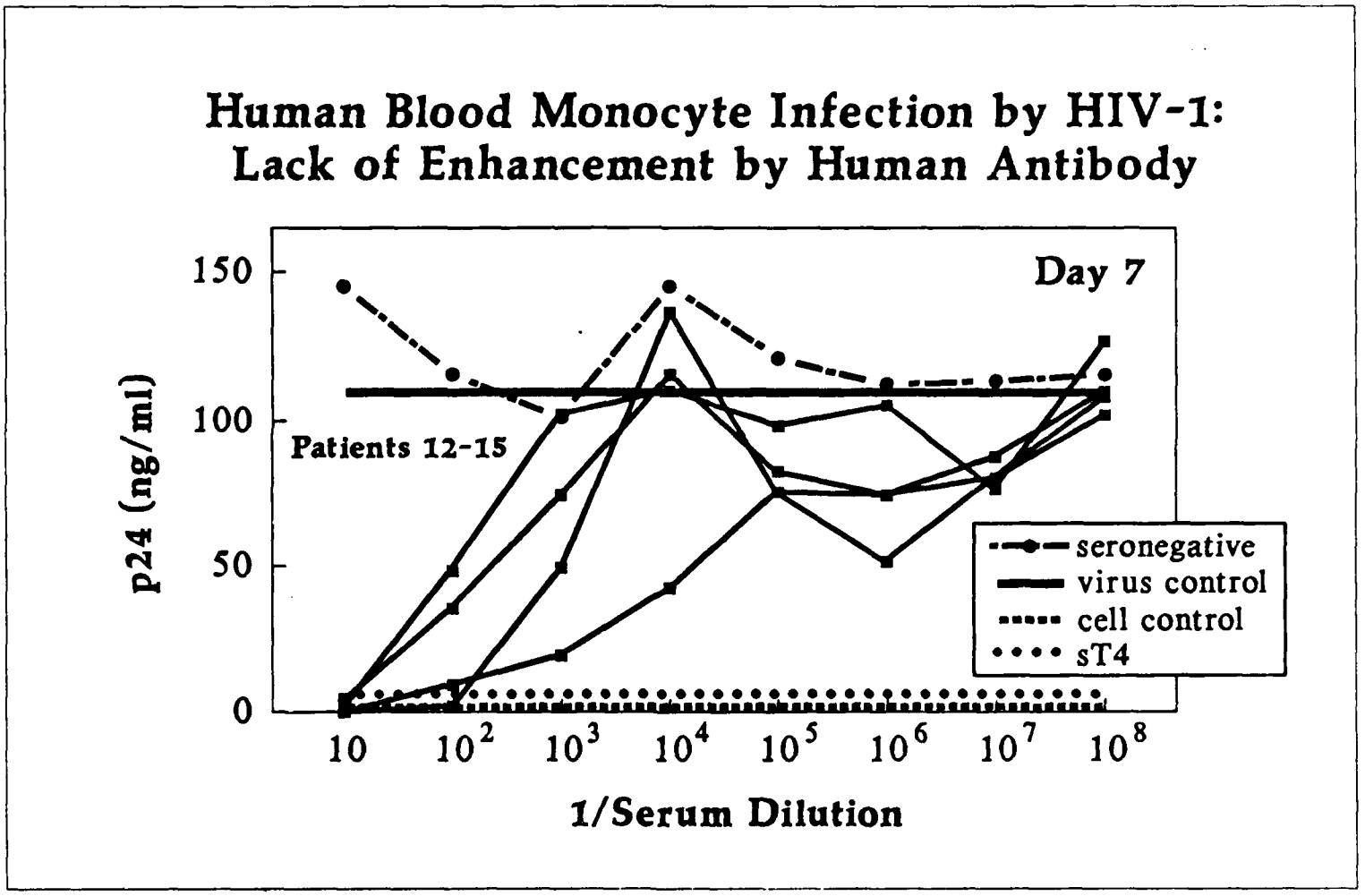


Figure 3. See legend of Figure 2 for explanation.

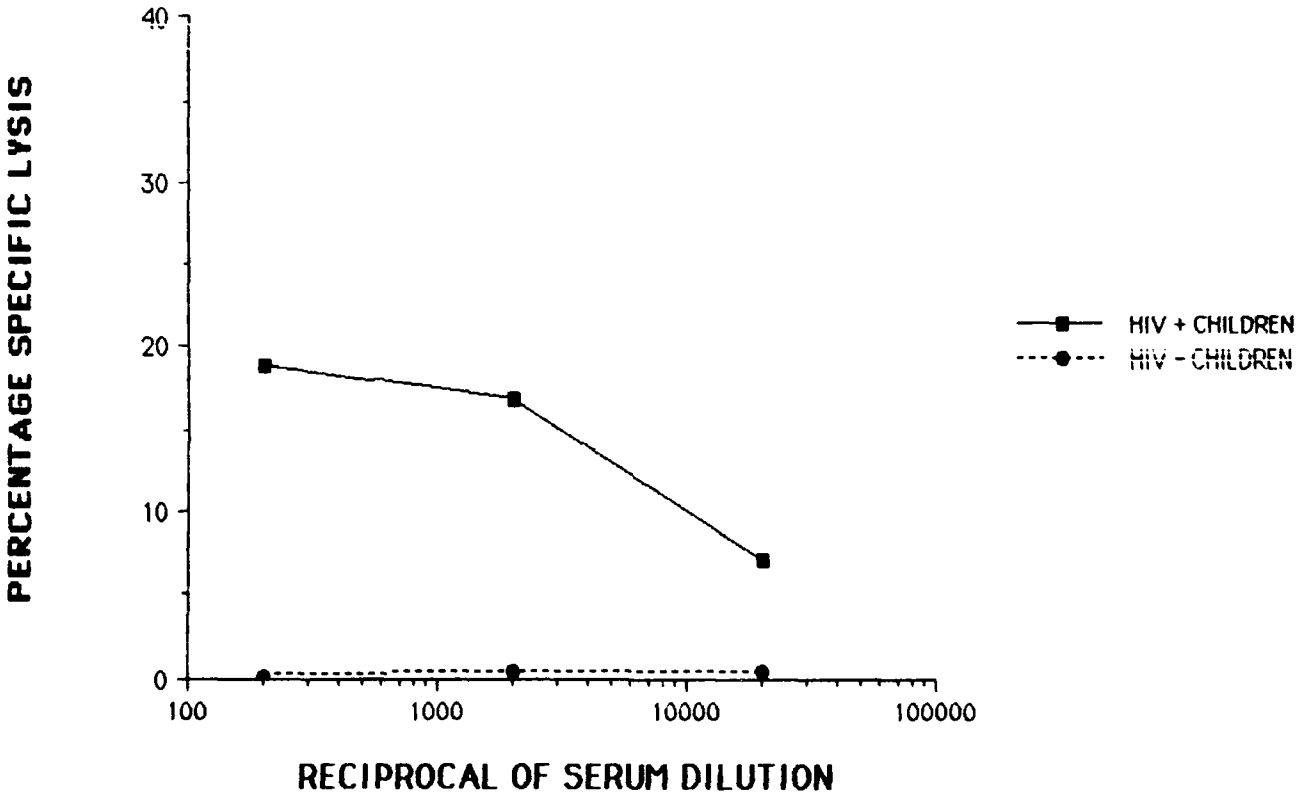


Figure 4. Anti-gp120 ADCC in HIV infected versus uninfected children.

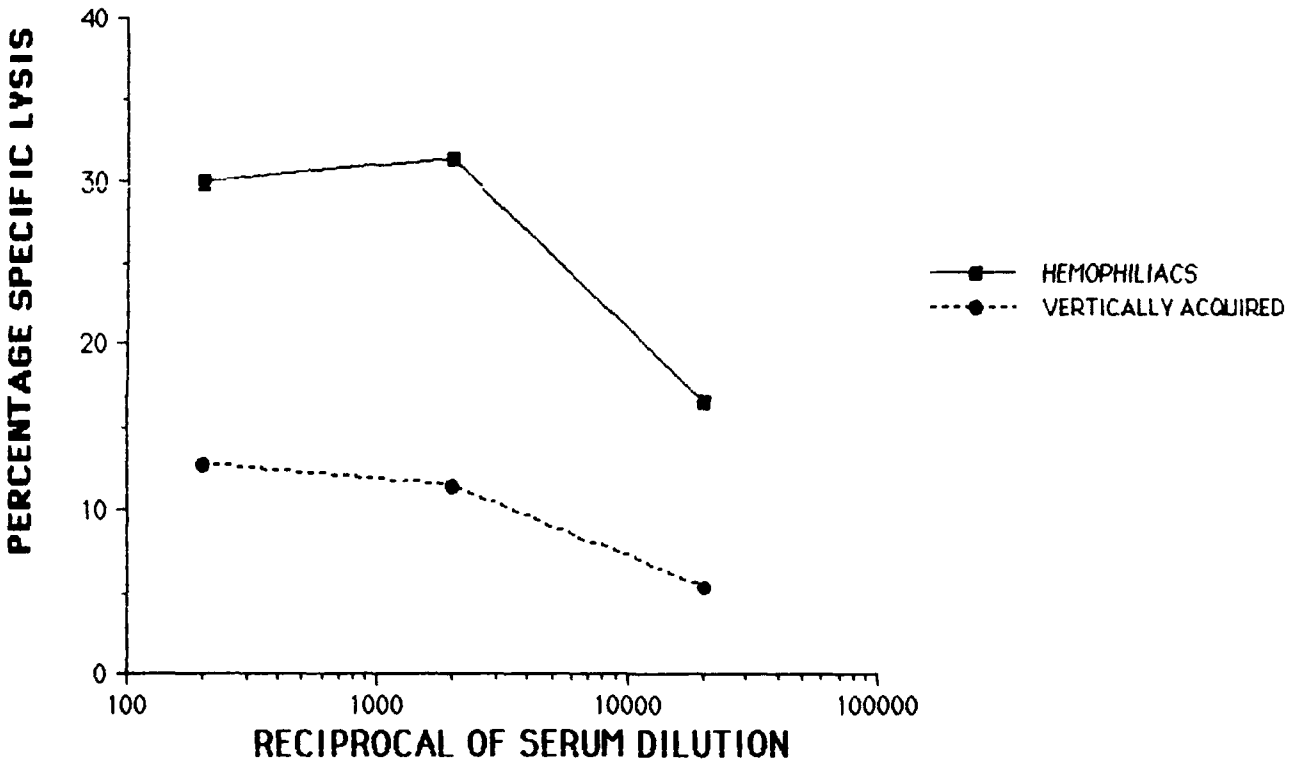


Figure 5. Anti-gp120 ADCC in HIV infected children: Hemophiliacs versus vertically acquired.

Publications:

1) Javaherian K, Langlois AJ, Silver S, Profy AT, LaRosa GJ, Bolognesi DP, Putney SD, Herlihy WC and Matthews TJ. Principal neutralizing domain of HIV-1 envelope protein is capable of eliciting broadly neutralizing antibodies. In press.

2) LaRosa GJ, Emini EA, Profy AT, Weinhold KJ, Langlois AJ, Boswell N, Shadduck PP, Lewis J, Karplus M, Halley LH, Bolognesi DP, Matthews, TJ and Putney SD. The HIV-1 principal neutralizing determinant contains conserved sequence and structural elements. In press.

3) Walter EB, McKinney RE, Wilfert CM, McMillan CW, and Weinhold KJ. Antibody-dependent cellular cytotoxicity in HIV-1 infected children. In preparation.

4) Walter EB, McKinney RE, Lane BA, Weinhold KJ and Wilfert CM. Interpretation of Western blots in HIV-1 infected children: Implications for prognosis and diagnosis. In preparation.

5) Shadduck PP, Weinberg JB, Haney AF, Bartlett JA, Langlois AJ, Bolognesi DP, and Matthews TJ. HIV-1 infection of human peritoneal macrophages and blood monocytes: Lack of enhancement by human antibody. In preparation.